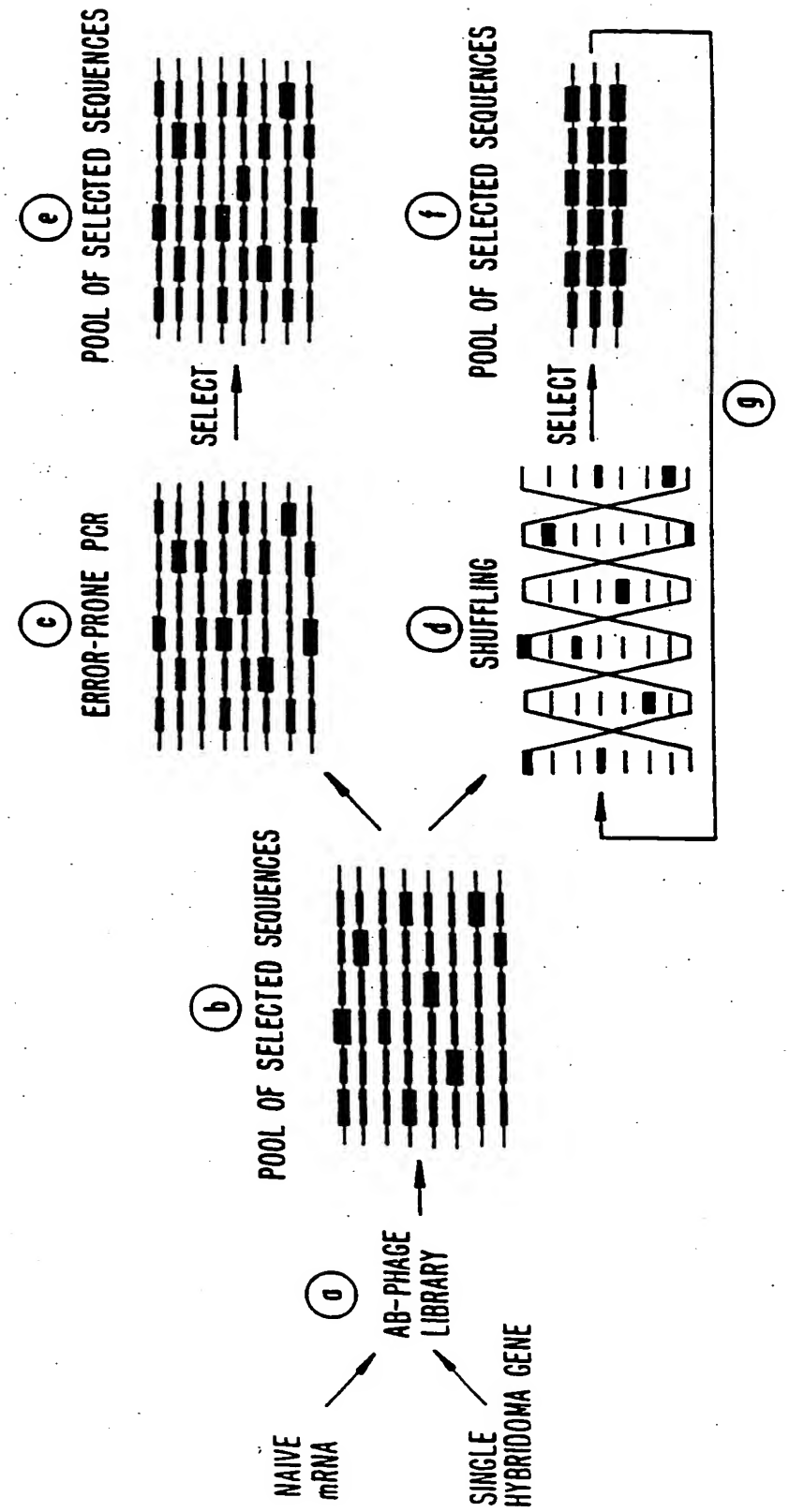
NAIVE mRNA
SINGLE HYBRIDOMA GENE
a
AB-PHAGE LIBRARY
b
POOL OF SELECTED SEQUENCES
c
ERROR-PRONE PCR
d
SHUFFLING
SELECT
e
POOL OF SELECTED SEQUENCES
SELECT
f
POOL OF SELECTED SEQUENCES
g

FIG. 1.

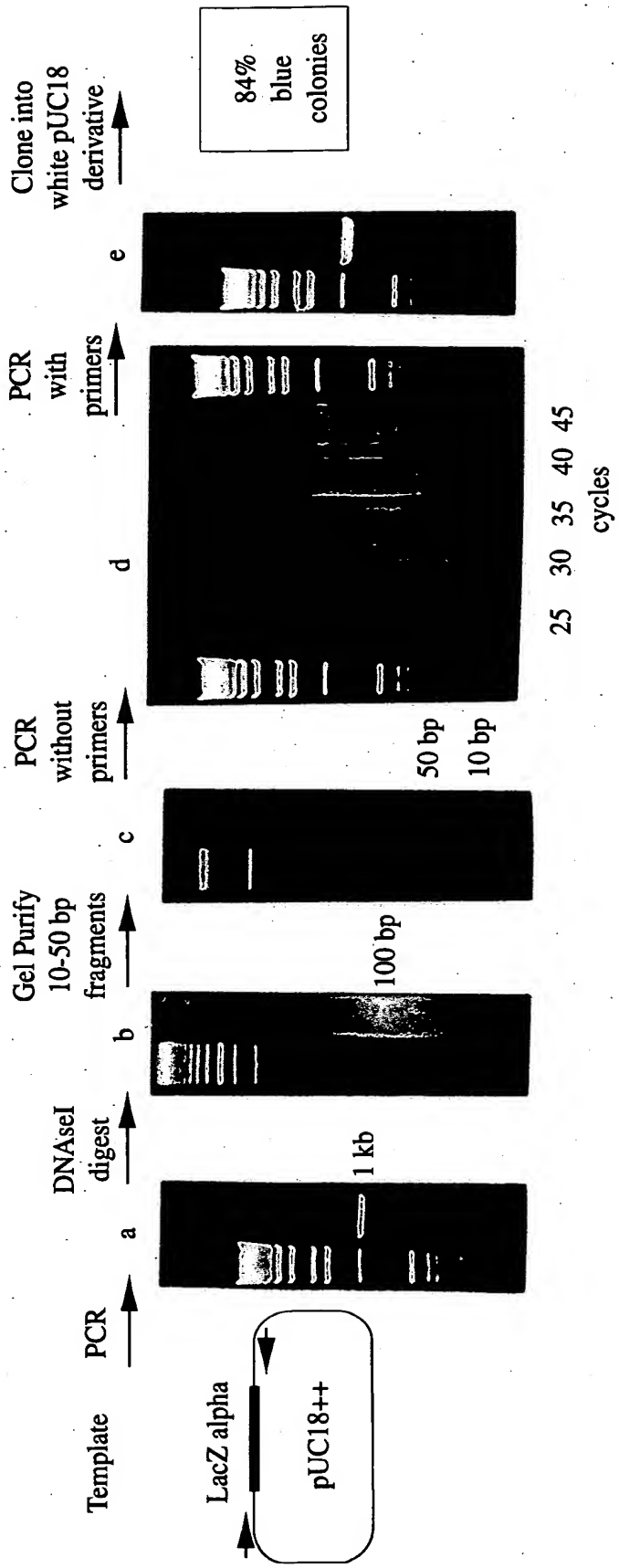
Template
PCR
LacZ alpha
pUC18++
a
1 kb
DNAseI digest
b
100 bp
Gel Purify 10-50 bp fragments
c
50 bp
10 bp
PCR without primers
d
25 30 35 40 45 cycles
PCR with primers
e
84% blue colonies
Clone into white pUC18 derivative

Figure 2

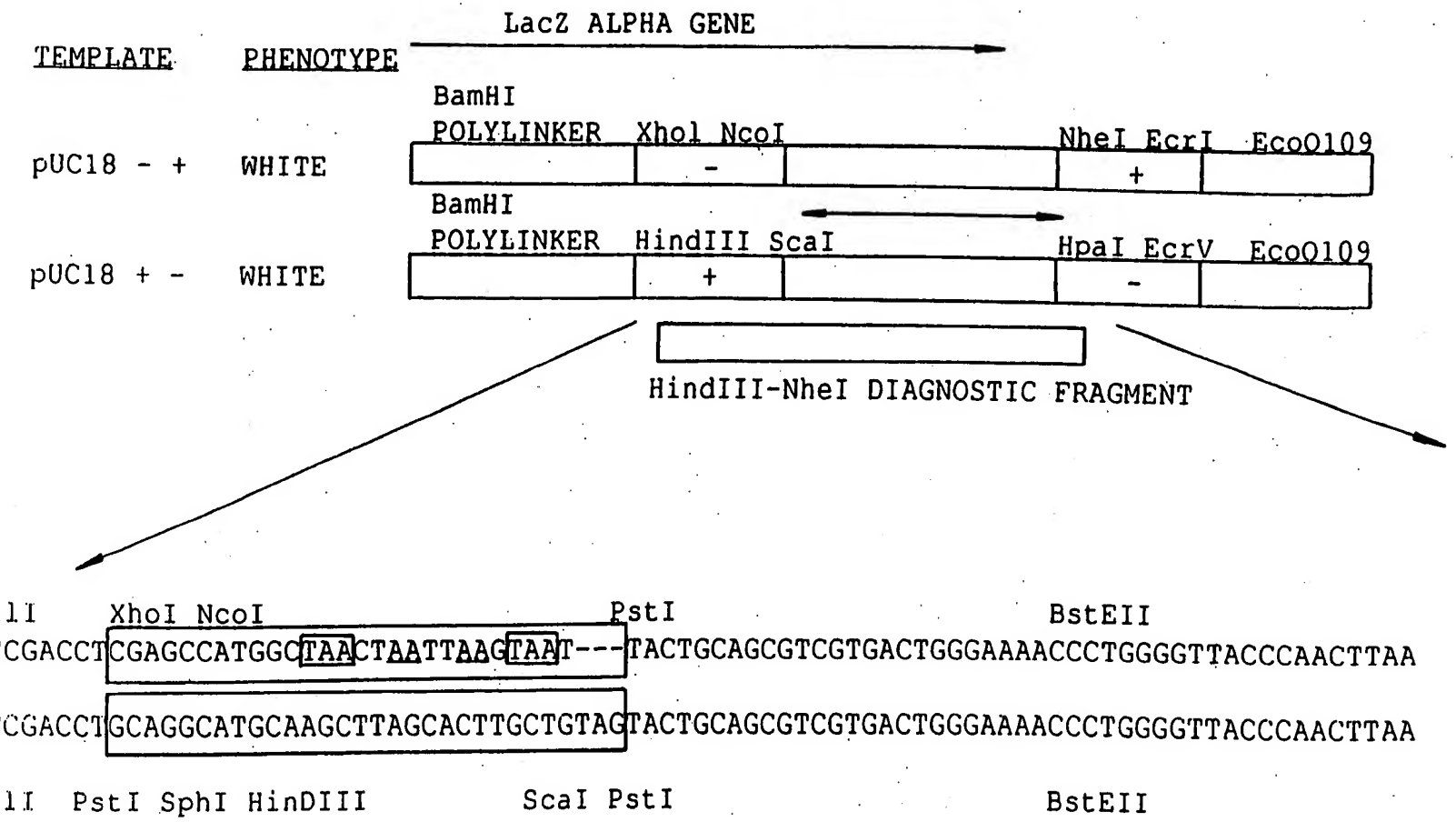

FIG. 3A.

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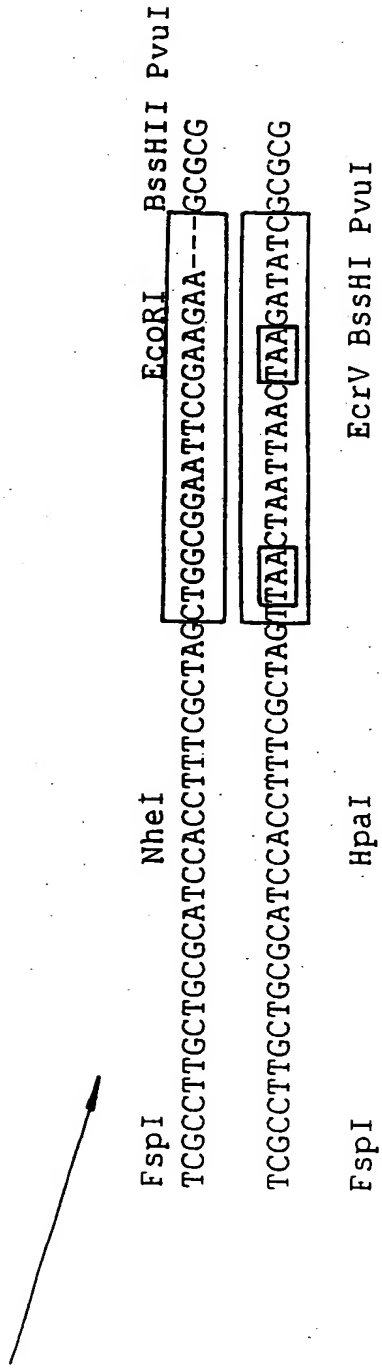

FspI
NheI
EcoRI
BssHII PvuI
TCGCCTTGCTGCGCATCCACCTTTCGCTAGCTGGCGGAATTCCGAAGAA---GCGCG
TCGCCTTGCTGCGCATCCACCTTTCGCTAGTTAACTAATTAACTAAGATATCGCGCG
FspI
HpaI
EcrV BssHI PvuI

FIG. 3B.

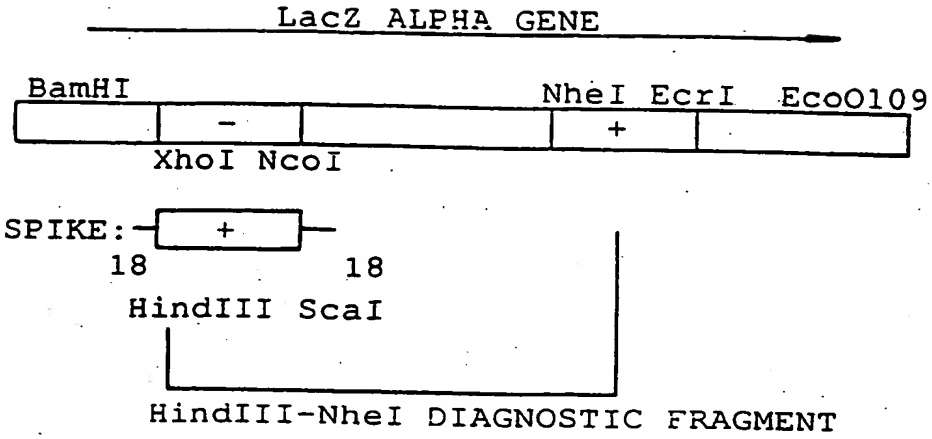

LacZ ALPHA GENE
BamHI
NheI EcrI Eco0109
-
+
XhoI NcoI
SPIKE:
+
18
18
HindIII ScaI
HindIII-NheI DIAGNOSTIC FRAGMENT

FIG. 4.

M ATGGTTCCGATCCGTCAGCTGCACTACCGTCTGCGTGACGAACAGCAGAAAAGCCTGGTTCTGTCCGACCCGTACGAACTGAAAGCT

II ATGGCACCGGTTAGATCTCTGAACTGCACCCTTCGCGACTCCCAACAGAAAAGCTTAGTAATGTCTGGTCCGTACGAGCTCAAAGCT

M AGGTGATCTTCTCCATGAGCTTCGTACAAGGTGAACCAAGCAACGACAAAATCCCGGTGGCTTTGGGTCTGAAAGGTAAAAACCTGT

II AGGTTGTATTCAGCATGAGCTTCGTCCAAGGTGAAGAGTCTAACGACAAGATCCCAGTTGCATTAGGCCTGAAAGAGAAGAATCTGT

M GACCCTGCAACTCGAGAGCGTGGACCCAAAACAGTACCCAAAGAAGAAGATGGAGAAGCGTTTCGTCTTCAACAAGATCGAAGTCAA

II GACTCTGCAGCTTGAATCCGTTGACCCGAAAAACTATCCGAAGAAGAAAATGGAGAAGCGTTTCGTATTTAACAAGATTGAGATTAA

M CCGAACTGGTACATCAGCACCTCCCAAGCAGAGCACAAGCCTGTCTTCCTGGG...TAACAACTCCGGTCAGGATATCATCGACTTC

II CCAAACTGGTACATCAGTACTTCTCAAGCAGAGAATAAGCCTGTGTTCCTCGGCGGTACCAAAGGCGGTCAGGATATCACTGACTTC

FIG. 5A.

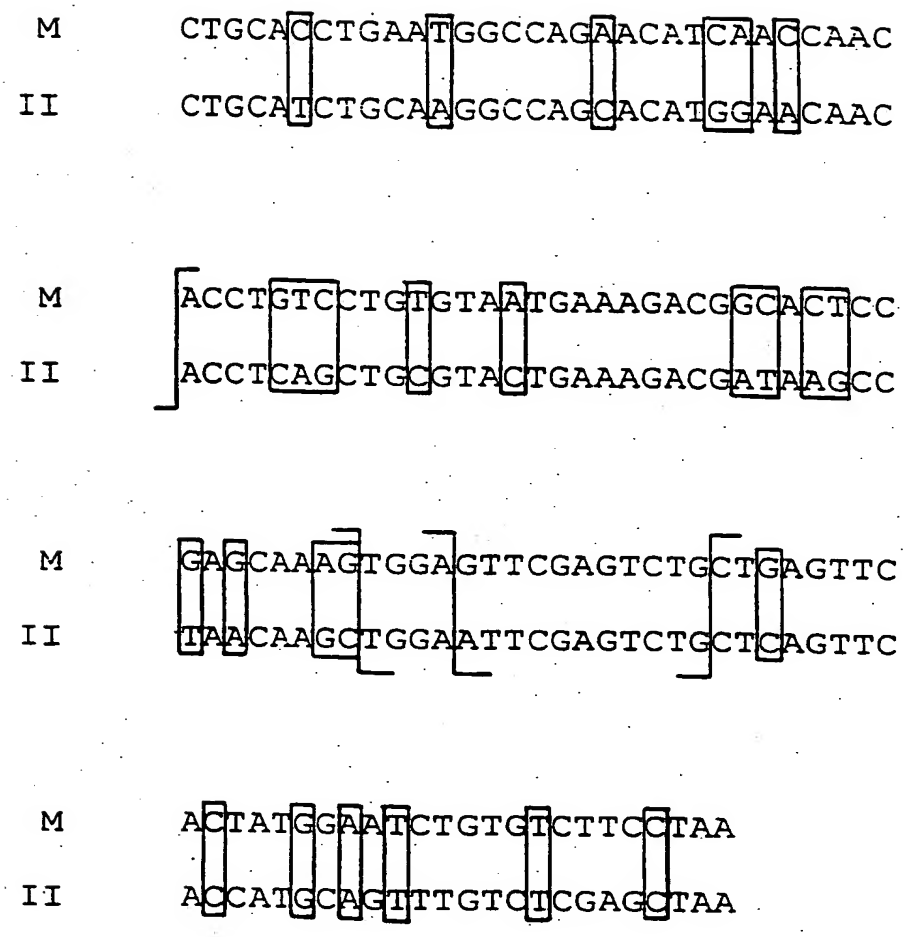
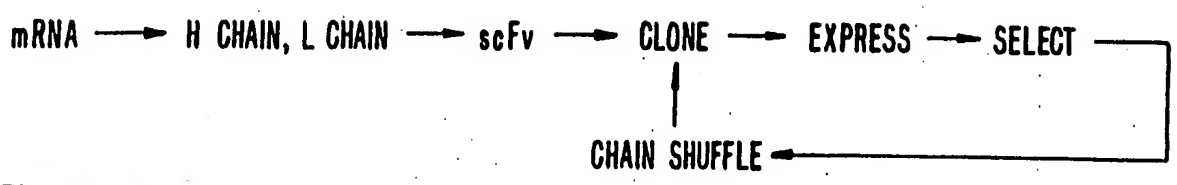
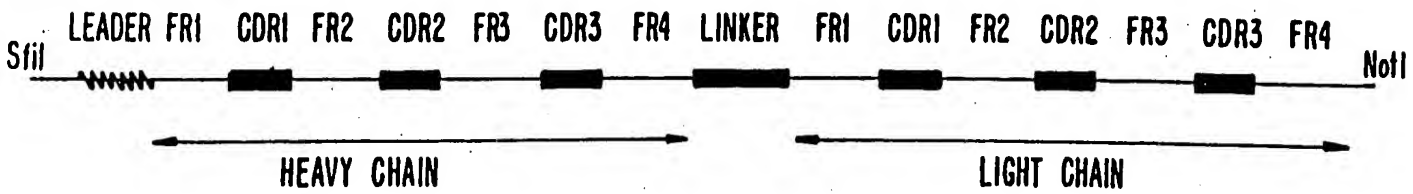

M CTGCACCTGAATGGCCAGAACATCAACCAAC
II CTGCATCTGCAAGGCCAGCACATGGAACAAC
M ACCTGTCCTGTGTAATGAAAGACGGCACTCC
II ACCTCAGCTGCGTACTGAAAGACGATAAGCC
M GAGCAAAGTGGAGTTCGAGTCTGCTGAGTTC
II TAACAAGCTGGAATTCGAGTCTGCTCAGTTC
M ACTATGGAATCTGTGTCTTCCTAA
II ACCATGCAGTTTGTCTCGAGCTAA

FIG. 5B.

A10B = scFv OF ANTI-R-IgG ANTIBODY (PHARMACIA)

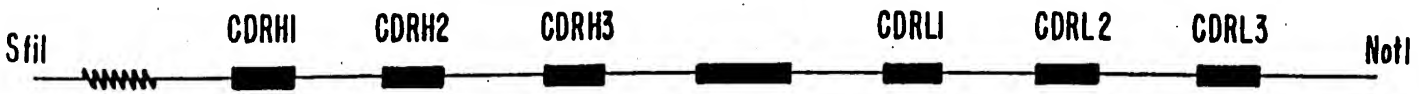


scFv STRUCTURE



TOTAL: 750 bp

FIRST EXPERIMENT: A10BscFv



SPIKE: 70/10/10/10CDRs:

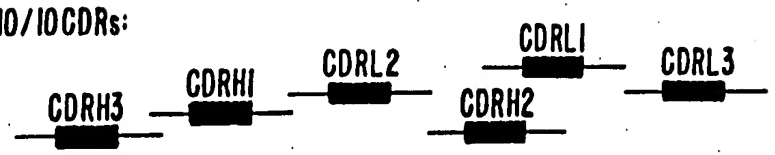


FIG. 6.

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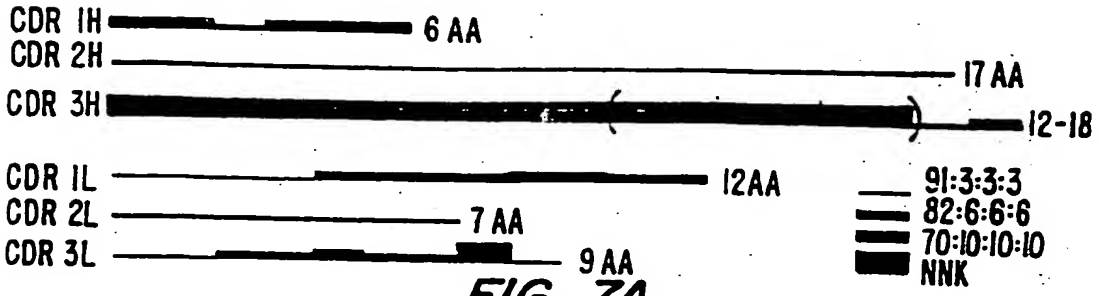


FIG. 7A.

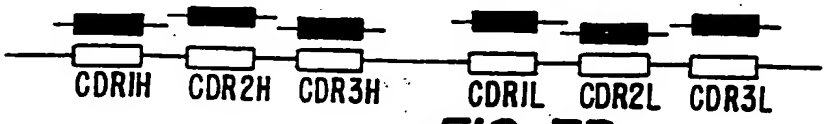


FIG. 7B.

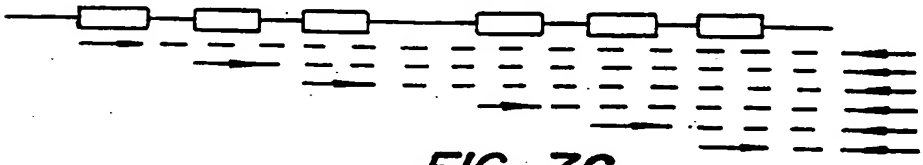


FIG. 7C.

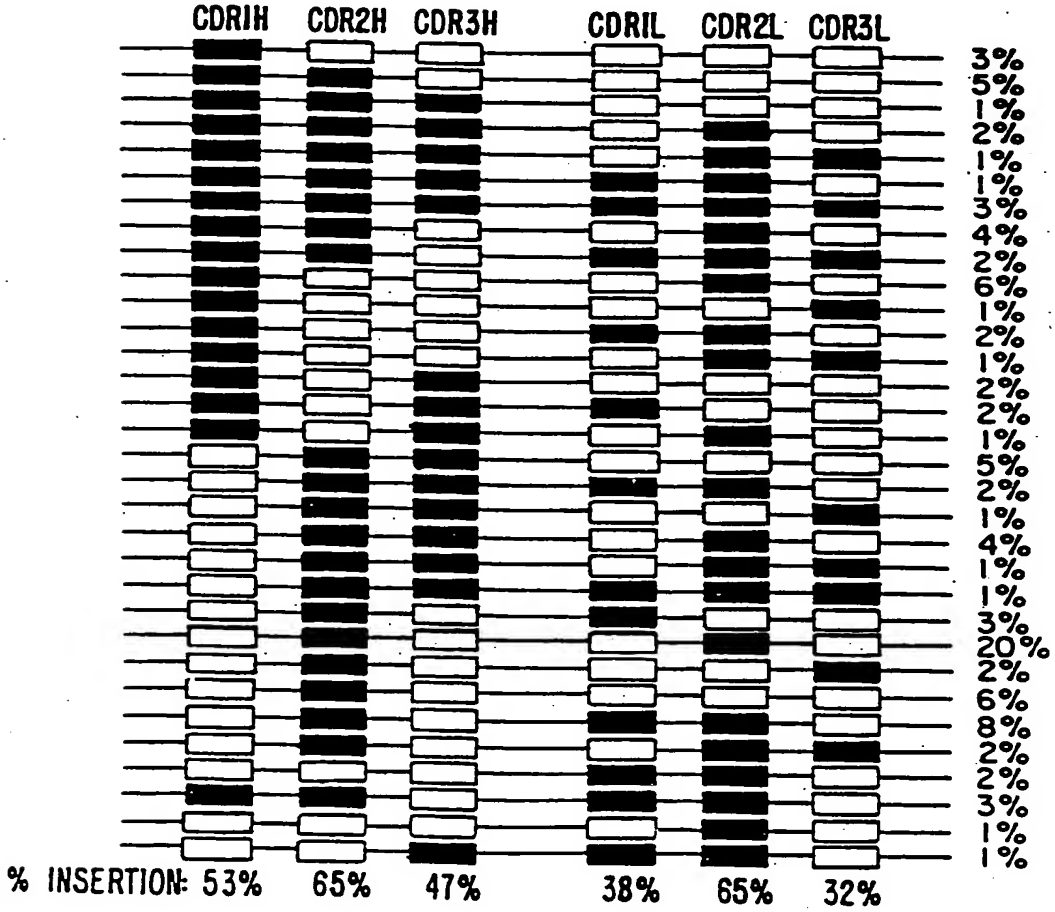


FIG. 7D.

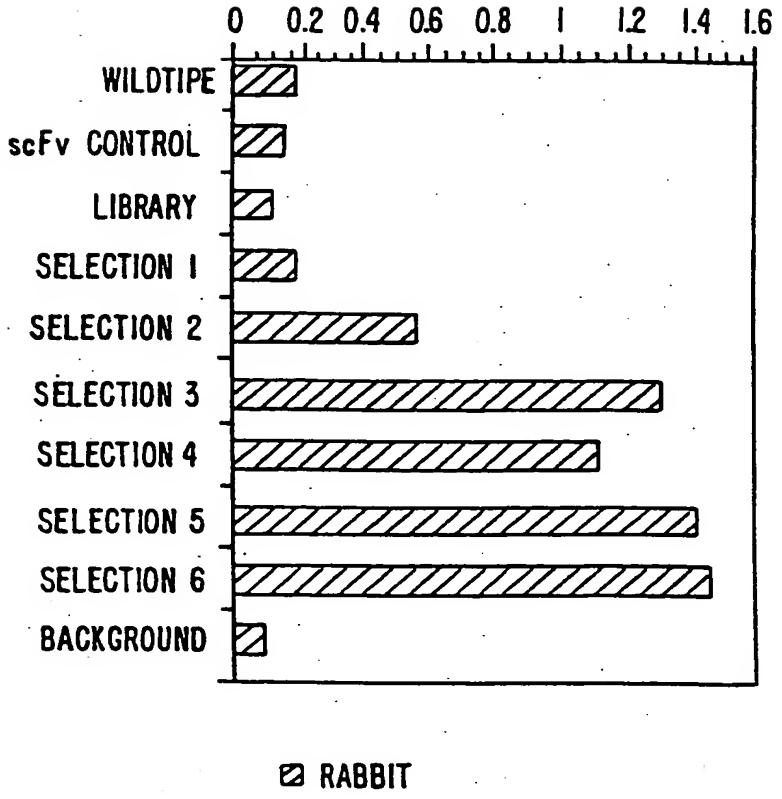
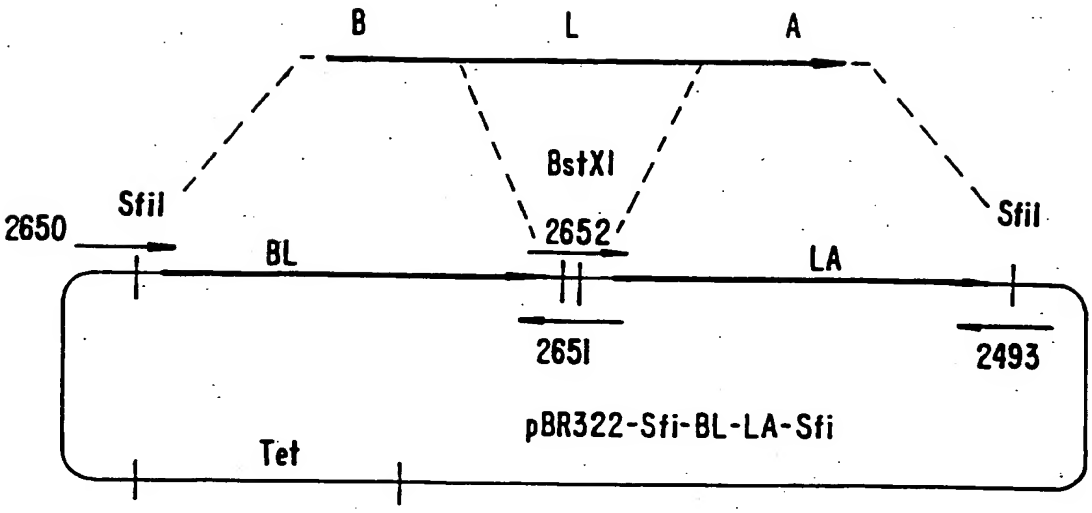
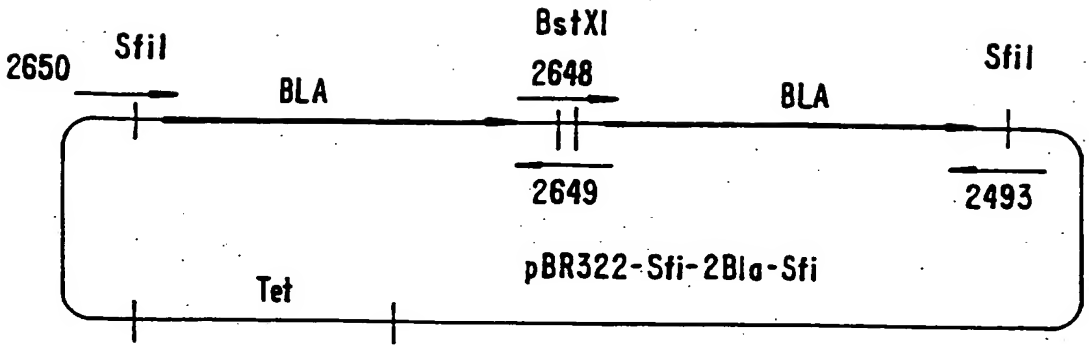

0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
WILDTIPE
scFv CONTROL
LIBRARY
SELECTION 1
SELECTION 2
SELECTION 3
SELECTION 4
SELECTION 5
SELECTION 6
BACKGROUND
RABBIT

FIG. 8.



CELL	Tet COLONIES	Amp COLONIES	COLONY PCR
TG-1	131	21	3/3 AT 1 KB
JC8679	123	31	4/4 AT 1 KB
VECTOR CONTROL	51	0	

FIG. 9.



CELL	Tet COLONIES	Amp COLONIES	COLONY PCR
TG-1	28	54	7/7 AT 1 KB
JC8679	149	117	3/3 AT 1 KB
VECTOR CONTROL	51	0	

FIG. 10.

APPROACH	AMP COLONIES	AMP TET COLONIES	% HOMOLOGOUS RECOMBINATION	COMMENT
1-CUT VECTOR 1 INSERT JC8679	4,000	1,500	100%(N=14)	EFFICIENT INSERTION BY HOMOLOGOUS RECOMBINATION WITH CO-ELECTROPORATED VECTOR
2-CUT VECTOR 2 INSERTS JC8679	2,000	16	100%(N=2)	100x LESS EFFICIENT THAN 1 FRAGMENT
3-UNCUT VECTOR 1 INSERT JC8679	16	0		HOMOLOGOUS INSERTION DEPENDS ON FREE ENDS
4-NO VECTOR 1 INSERT JC8679::pUCSfi-Sfi	5,000	10,000	70%(N=7)	IF VECTOR IS IN CELLS ALREADY, HIGH EFFICIENCY OCCURS EVEN THROUGH VECTOR IS UNCUT
5-NO VECTOR 1 INSERT JC8679	2,000	0		-CONTROL: NON-HOMOLOGOUS INSERTION INTO CHROMOSOME
6-CUT VECTOR NO INSERT JC8679	N.D.	0		-CONTROL: NO AMP BACKGROUND

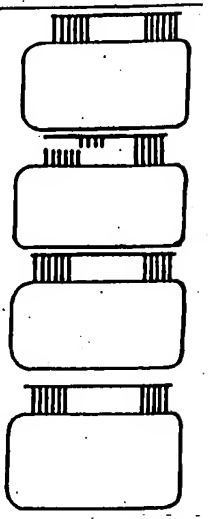


FIG. 11A.

HOMOLOGOUS RECOMBINATION COLONY PCR:

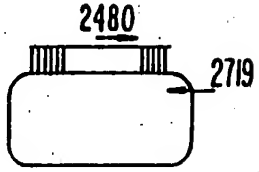


FIG. 11B.

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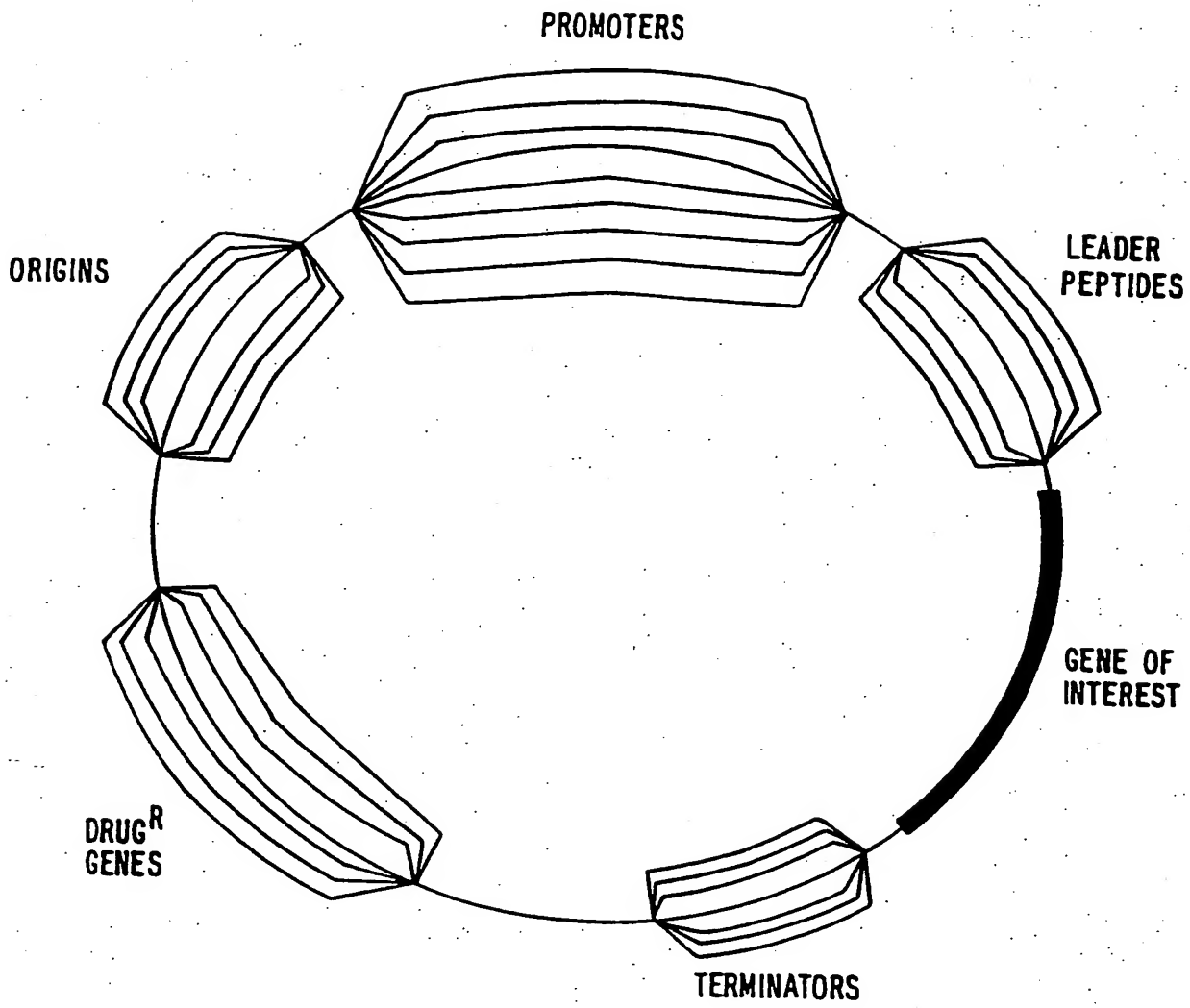
PROMOTERS
LEADER PEPTIDES
GENE OF INTEREST
TERMINATORS
DRUG^{R} GENES
ORIGINS

FIG. 12.

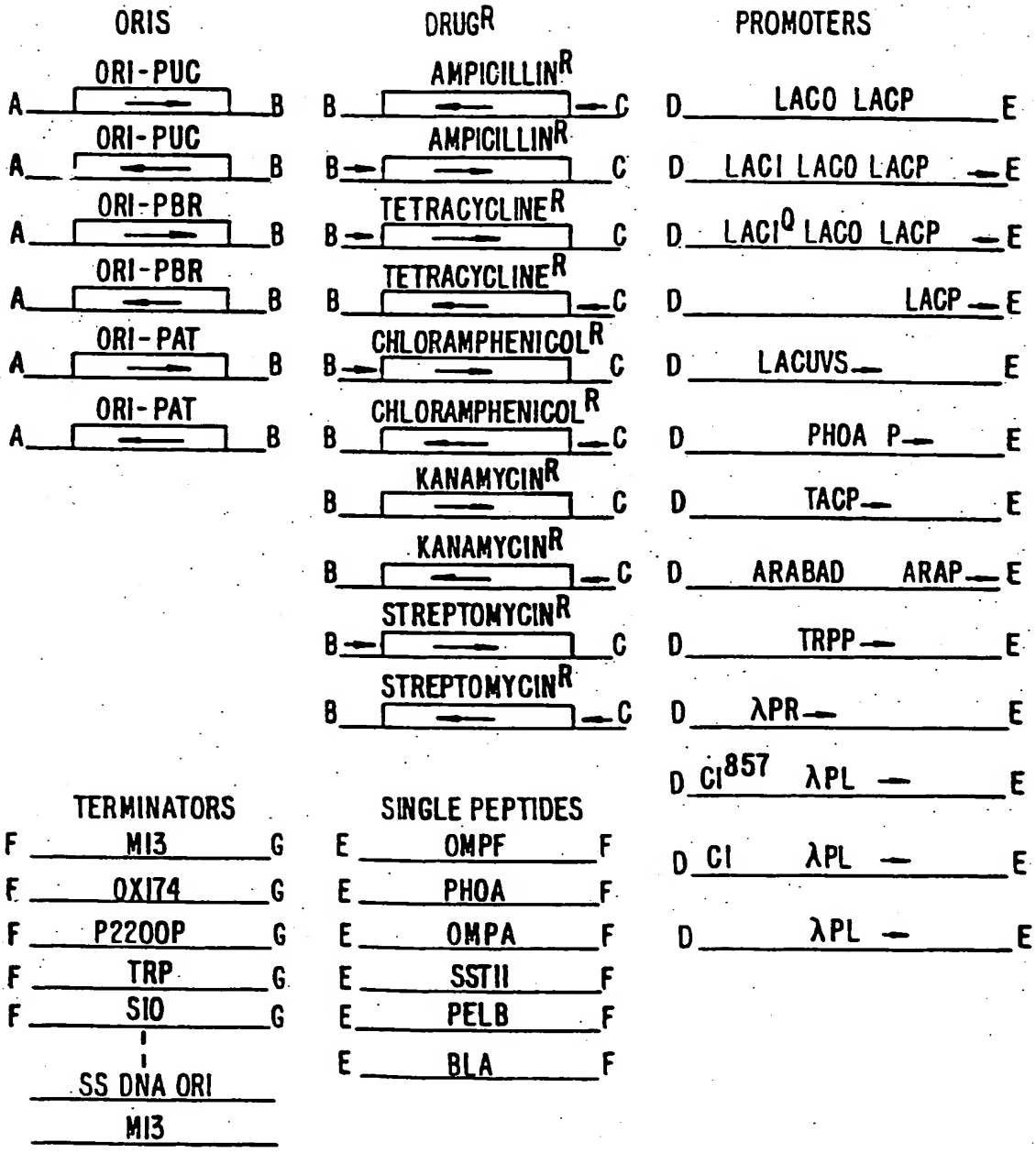
ORIS
ORI-PUC
ORI-PUC
ORI-PBR
ORI-PBR
ORI-PAT
ORI-PAT
DRUG^R
AMPICILLIN^R
AMPICILLIN^R
TETRACYCLINE^R
TETRACYCLINE^R
CHLORAMPHENICOL^R
CHLORAMPHENICOL^R
KANAMYCIN^R
KANAMYCIN^R
STREPTOMYCIN^R
STREPTOMYCIN^R
PROMOTERS
LACO LACP
LACI LACO LACP
LACI^Q LACO LACP
LACP
LACUVS
PHOA P
TACP
ARABAD ARAP
TRPP
λPR
CI857 λPL
CI λPL
λPL
TERMINATORS
M13
0X174
P2200P
TRP
S10
SS DNA ORI
M13
SINGLE PEPTIDES
OMPF
PHOA
OMPA
SSTII
PELB
BLA

FIG. 13.